
Hybrid median filter background estimator for correcting distortions in microtiter plate data.

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Public Summary:

Scientific Abstract:

Microtiter plate (MTP) assays often exhibit distortions, such as caused by edge-dependent drying and robotic fluid handling variation. Distortions vary by assay system but can have both systematic patterns (predictable from plate to plate) and random (sporadic and unpredictable) components. Random errors can be especially difficult to resolve by assay optimization alone, and postassay algorithms reported to date have smoothing effects that often blunt hits. We implemented a 5 x 5 bidirectional hybrid median filter (HMF) as a local background estimator to scale each data point to the MTP global background median and compared it with a recently described Discrete Fourier Transform (DFT) technique for correcting errors on computationally and experimentally generated MTP datasets. Experimental data were generated from a 384-well format fluorescent bioassay using cells engineered to express eGFP and DsRED. MTP arrays were produced with and without control treatments used to simulate hits in random wells. The HMF demonstrated the greatest improvements in MTP coefficients of variation and dynamic range (defined by the ratio of average hit amplitude to standard deviation, SD) for all synthetic and experimental MTPs examined. After HMF application to a MTP of eGFP signal from mouse insulinoma (MIN6) cells obtained by a plate-reader, the assay coefficient of variation (CV) decreased from 8.0% in the raw dataset to 5.1% and the hit amplitudes were reduced by only 1% while the DFT method increased the CV by 36.0% and reduced the hit amplitude by 21%. Thus, our results show that the bidirectional HMF provides superior corrections of MTP data distortions while at the same time preserving hit amplitudes and improving dynamic range. The software to perform hybrid median filter MTP corrections is available at http://bccg.burnham.org/HTS/HMF_Download_Page.aspx, password is pbushway.

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